The treatment of the Zygomycetes is substantially the same as for the preceding series: first, fourteen pages outlining the main features of the order Mucorinæ, then a key to the genera, followed by a description of the species of the genus Mucor as far as the end of the first section, Mono-Mucor.

This volume, while devoted to the forms occurring in Germany, Austria, and Switzerland, can not fail to be of great service to American students, since many of the described species occur in this country. Reference to doubtful forms and extra-European ones also help to make the book indispensible.—ERWIN F. SMITH.

Fruit culture in foreign countries.—Reports from the consuls of the United States on fruit culture in their several districts in answer to a circular from the Department of State. Washington, Government Printing Office, 1890, pp. 391–937; Index, i–xiii.

This report is devoted principally to the citrous fruits, the olive, fig, and vine. Incidentally there are many references to the diseases of these plants, parasitic and nonparasitic. Some of the statements need to be taken *cum grano salis* because emanating from men not specially trained to observations of this kind, but on the whole the reports appear to be well written and will prove useful. A similar volume on the stone fruits of the world would be equally valuable.—ERWIN F. SMITH.

MANGIN, LOUIS.—(1) *Sur la callose, nouvelle substance fondamentale existant dans la membrane.* Comptes Rendus, Paris, tome CX, 24 Mars, 1890, p. 644.

(2) *Sur les réactifs colorants des substances fondamentales de la membrane.* Comptes Rendus, Paris, tome CXI, 15 Juillet, 1890, p. 120.

(3) *Sur la structure des Péronosporées.* Comptes Rendus, Paris, 15 Décembre, 1890, p. 923.

(4) *Sur la désarticulation des conidies chez les Péronosporées.* Bull. de la Soc. Bot. de France. Comptes Rendus des Séances, Paris, 1891, tome 38, pp. 176–184, 232–236, pl. 4.

(1) The author distinguishes three fundamental substances in the cell walls of plants—pectin compounds, cellulose, and callose. The latter has been studied quite carefully, and is described as a new fundamental substance, known hitherto only from sieve tubes. Not having been able to isolate it in sufficient purity for a chemical analysis, the author confines himself to an account of its distribution in plants.

Callose is colorless and amorphous, insoluble in water, alcohol, and Schweizer's reagent,* even after the action of acids; very soluble in soda or cold caustic potash 1 to 100, soluble cold in sulphuric acid, chloride of calcium, and concentrated bichloride of tin; insoluble cold in the alkaline carbonates, and in ammonia, which swells it and gives it a gelatinous consistency. Besides aniline blue and rosolic acid†

*Cuprammonia. †Known also as corallin, aurin, peonin.

already recommended by Russow and Janczewski for the study of liber, the color reagents of callose are certain substances of the series of azo colors, belonging to the group of benzidines, tolidenes, etc. Iodated reagents give to callose a yellow tint. Callose is therefore as distinct as cellulose or the pectin compounds. It is not a result of the artificial decomposition of the latter substances, for these may be treated in all sorts of ways without producing the reactions of callose. Its insolubility in the cuprammoniacal reagent, even after the action of acids, and the yellow color which it gives with iodated phosphoric acid distinguish it from cellulose, while its insolubility in cold ammonia and alkaline carbonates, and its inertia toward stains which act on the pectin compounds separate it not less clearly from the latter.

While callose exists normally in certain regions of the reproductive organs of phanærogams (pollen grains, pollen tubes, etc.) and vascular cryptograms, it is not found in the vegetative portions of these plants, exclusive of the liber, save accidentally and as irregular masses scattered through the cells. But in the thallophytes callose acquires a great importance. In the fungi it forms the membrane of the mycelium and of the organs of fructification in the most widely separated families; *e. g.*, Peronosporeæ, Saprolegniaceæ, Basidiomycetes, Ascomycetes, Saccharomycetes. In lichens callose exists in the mycelial filaments, but not in the gonidia. It does occur, however, in some of the algæ. On the other hand, he has not yet found it in certain Uredineæ, nor in the mycelium and conidiophores of the Mucorinæ. In the plants of this order Mucor, Phycomycetes, Rhizopus, Pilobolus, Chætocladium, etc., it constitutes only the dissolving wall of the sporangium, and some part of the membrane of the spores. Callose appears to be in a state of purity in the membrane of the sporangium of the Mucorinæ, but in the mycelium of the Peronosporinæ and Saprolegninæ it is intimately united with cellulose, to the exclusion of pectin compounds, and, finally, in the Polyporei (Dædalea), the mycelial tubes appear to be destitute of cellulose, and are formed of callose associated with substances having the reaction of pectin compounds.

Various circumstances often mask the presence of callose, such as physical differences or the incrustation of foreign substances, for example, the callose of pollen mother cells and that which forms irregular masses in the mycelium and haustoria of the Peronosporinæ presents the most alterable and easily distinguishable state. In the sporangium of the Mucorinæ and the mycelium of lichens the callose offers more resistance to the action of solvents and fixes stains less readily. Finally, in the Polyporei it coheres so strongly that its presence can be demonstrated only after long and repeated treatments.

(2) The various stains of the aromatic series may be divided into two groups, one consisting of basic colors united with various mineral or organic acids, the other of acid colors used in the form of alkaline salts. Substances of the first category are fixed with a variable energy

by pectin compounds, which thus reveal their acid function. They do not stain callose or cellulose. The following compounds are noteworthy: *Azo group* Vesuvin brown, chrysoidin; *diphenyl-methane group*, auramine; *triphenylmethane group*, the Victoria blues, bleu de nuit, fuchsine, Paris violet, Hofman's violet, etc., all the stains of the *oxazine group*, naphthaline blue, Nile blue; *thionine group*, methyl blue; *euhrodine group*, neutral red; *safranine group*, neutral blue, pheno-safranine extra safranine, rosaline, Magdala red. The affinity of these substances for pectin compounds is very dissimilar. It is also feeble, for the presence of an excess of acid or of glycerine removes the stain from the tissues more or less readily.

The second category, formed of alkaline salts, contains a great number of substances which never stain pectin compounds. Many, however, are fixed by cellulose and callose, and thus show the basic nature of these latter, a nature already known and used for a long time, so far as concerns cellulose. In this category only two groups are of interest, the *azo group* and the *triphenylmethane group*. The azo group, exclusive of chrysoïdine and Vesuvin brown, is composed principally of alkaline salts. In this group we distinguish three important types. The first includes the various stains which contain the azo grouping once, *e. g.*, xylidine ponceau, aniline ponceau, toluidine ponceau, naphthorubin, etc., as well as various tropeolines of a slightly different composition. These substances stain protoplasm yellow, but they have no action on cellulose and callose. The second type is formed of substances containing the azo grouping twice, *e. g.*, orseille red, orseilline BB, azorubine, naphthol black, the croceines, etc. These substances stain cellulose in a neutral or slightly acid bath, but have no effect on callose. The third type contains the stains of the benzidine series, *e. g.* Congo red, Congo GR, Congo brilliant G, Corinthian Congo, extra Bordeaux, delta purpurine G, which result from the action of sulphonated naphthol compounds upon benzidine; azo blue, Corinthian Congo B, the benzo purpurines and the rosazurines, in which toluidine is substituted for benzidine; azo violet, the benzo azurines and heliotrope, where dianisidine is substituted for benzidine. These colors, ordinarily precipitated by acids, stain cellulose directly in a neutral or, better, a slightly alkaline bath.

The triphenylmethane group does not offer as distinct relations between staining capacity and chemical composition. We first distinguish a large number of bodies formed by chlorhydrates, sulphates, etc., which stain pectin compounds directly. Then a series of alkaline salts which may be divided into three groups. The first group includes acid fuchsine, acid violet, Bayer's blue, the alkaline blues, etc., which result from the respective action of sulphuric acid on fuchsine, Paris violet 6B, diphenylamine blue, and analine blue. These substances do not stain cellulose, but certain of them stain callose, *e. g.*, the soluble blues, and notably Bayer's blues. The staining is energetic

in proportion to the completeness of the sulphonization, *e. g.*, the blue 6B, a mixture in which trisulphonated triphenylrosanilin predominates, is the most active of the soluble blues. The second group is formed by the alkaline salts of rosolic acid, which stain callose and cellulose directly. Finally, the third group, the eosines, or salts or fluorescine such as eosine, erythrosine, and phloxine, stain nitrogenous matters deeply, but are not fixed by callose or cellulose.

As various stains of the aromatic series also combine with nitrogenous substances, to avoid error it is often indispensable that there should be a mixture of several reagents belonging to different categories. This gives a very demonstrative double stain.

(3) The constitution of the membrane of fungi is still unknown. The author believes that fungine and metacellulose do not exist as specifically distinct substances. The membrane of fungi is so complex and variable that it would be possible to offer the chemical composition in evidence whenever the absence of fructification rendered the determination of families uncertain.

In the group under consideration the membrane is composed of callose and cellulose closely associated. To show this, leaves containing *Peronospora ficariæ* may be treated as follows:

(*a*) Treat with concentrated chlorhydric acid; (*b*) macerate for some minutes in Schweizer's reagent. This removes all the cellulose and pectin compounds contained in the host and in the parasite. After washing in water, the use of iodated phosphoric acid or of the benzidine colors does not reveal a trace of cellulose in the tissue of the leaf, but the reagents of callose bring out a network of mycelial filaments. Contrarywise, if we submit the contaminated leaves of the Ranunculus to the action of Hofmeisters's chlorated mixture* and after washing allow the tissues to macerate in a solution of potassa or caustic soda, renewed several times, all the callose is removed without sensible modification of the cellulose. Then by the use of iodated reagents we can see the mycelial filaments stained blue or violet in the midst of the disassociated tissues of the host plant.

Thus either the cellulose or the callose can be removed without changing the form and arrangement of the mycelium. But while cellulose and callose are always associated in the organs which the parasite sends into the host (mycelium and oöspores), the conidiophores are formed of pure cellulose. This is proved by their disappearance after the action of cellulose solvents.

The mycelial membrane varies in thickness and shows numerous layers, but what gives the mycelium of the Peronosporinæ a special character is the constant presence of masses of callose, which is either pure or associated with cellulose. These constrict the cavity of the tube or even obliterate it. In the latter case, they form the so-called septa. These masses are seen very clearly in *Peronospora parasitica*, *P. Schleideni*, *P. myosotidis*, *Plasmopara viticola*, etc. They serve very clearly

to distinguish the Peronosporinæ from other parasites. Pollen tubes inside of tissues are the only bodies likely to be confounded with them, and this only in case of species with much reduced haustoria.

The haustoria have the same structure as the mycelium and their shape and varying size always furnish excellent data for distinction of species. They are sometimes so minute as to have thus far escaped the attention of botanists, *e. g.*, *Phytophthora infestans*, described in all the books as destitute of haustoria, possesses numerous ones which are extremely minute and filiform. Haustoria are simple or branched: (*a*) Simple and oval or spherical (*Cystopus candidus*, *Plasmopara viticola*. *Pl. epilobii*, *Peronospora leptosperma*, etc.); (*b*) Claviform and simple (*Bremia lactucæ*); (*c*) filiform and simple (*P. myosotidis*, *P. Schleideni*, *P. affinis*, *P. chlorœ*, *Phytophora infestans*); (*d*) ramified and claviform (*P. parasitica*); (*e*) ramified and filiform (*P. arborescens*, *P. calotheca*, etc.). Ordinarily the haustoria have a double envelope and between these two envelopes irregular and voluminous masses of callose often occur and sometimes rupture the exterior membrane (*Cystopus candidus*, *P. myosotidis*, etc.). At other times the exterior membrane shows only cellulose and incloses little callose. It then forms a complete sheath around the haustorium which can be removed in connection with the mycelium by a slight traction (*P. Schleideni*). Sometimes the masses of callose formed by the haustoria are so abundant that they fill the entire cavity of the cell, the protoplasm being crowded against the wall.

Masses of callose in a state of purity are also found in the cavity of the conidiophores. They take the form of rings or irregular plugs, of most variable location. In any case these plugs can not be likened to the septa which form at the base of the sporangium of the Mucorinæ, as has been done. The only part of the conidiophores where the presence of callose is constant is the base of the conidia where this substance plays an important role in the dissemination of the spores.

To sum up, the constant presence of callose in the mycelium of the Peronosporinæ enables us to recognize with great clearness the least traces of these parasites in the host plants and to show clearly the relations which exist between the latter and the parasite.

(4) This paper is really a continuation of the last one. Observations on the formation and the separation of the conidia in *Cystopus candidus* led M. Mangin to the following conclusions: The septum first appears as a delicate ring of callose on the thin inner wall of the basidium. This ring gradually enlarges until only a small central opening remains. The septum then appears as a funnel minus its tube, the convexity of which projects toward the base of the basidium. The open central portion of the septum finally closes. About this time or a little sooner the thin cellulose wall of the basidium disappears at the level of the callose (is absorbed) and a constriction rapidly takes place, the base of the new conidium and the summit of the basidium rounding off by the

extension and growth of the cellulose membrane. The conidium is now attached to the basidium by a mass of callose in the form of a little cup embracing the slightly pyriform base of the conidium. The base of this cup is convex or plane, but the center often shows a little pit which is the last vestige of the previous funnel-form orifice.

No division of the connecting cupule into three layers, as described by Zalewski, was observed. At this stage it is pure callose. Soon the cupule contracts, its superior edges being reduced progressively, and it shortly takes on the form of a cylindric fragment uniting the conidia, but the cellulose membrane of the conidium or of the basidium is not yet continuous across the callose. Subsquently the cellulose wall of the upper part of the basidium is continued along the base of the callose plug or through it when the latter projects. A similar process takes place a little later at the lower end of the conidium when the cup form has almost disappeared. Sometimes this cross wall is outside of the callose band, sometimes it grows through it, imprisoning a portion within the conidium. It is generally only when the conidium is second in rank from the basidium that the cellulose membrane is completed. Up to this point the changes in the callose band have been due to absorption, but not so subsequently. The band of callose now changes chemically so as to become strongly hygroscopic and completely soluble in water or even in the vapor of water. This primary septum or connective band contains no pectin compounds and does not swell and become gelatinous previous to solution, as stated by de Bary and Zalewski. It is simply a very neat case of liquefaction.

New conidia are developed under the old ones in the following manner: The end of the basidium elongates by intercalary growth and a new ring of callose appears. This is not always in the same plane, but most often for each conidium or group of conidia it appears in a region nearer the summit, so that the lateral wall of the basidium presents a series of thickenings, which when stained appear as striæ. Each one of these striæ corresponds to a group of conidia, for they are always less numerous than the conidia successively developed from a basidium.

The formation of conidia finally ceases, and in old sori, long ruptured, it is easy to find such exhausted basidia drawn out to a naked point or terminated by a single conidium which appears to be incapable of completing its development. The membrane of the basidium is then notably thickened in the terminal region and more or less deformed. The striæ above mentioned are often visible and, finally, several rows of internal button-shaped thickenings are almost always present. These thickenings are composed either of pure callose or of a mixture of callose and cellulose.

The statements here given were also found to hold good for *Cystopus cubicus* and the closely related *C. spinulosus*. A somewhat similar method of growth and delimitation was studied in a form of *Plasmopara* found on *Epilobium montanum*. Here, however, the cellulose wall

at the base of the spore is reflected over the upper surface of the callose somewhat early, but fades out toward the center. At this time the extremity of the basidium is expanded funnel-form, and the callose septum is biconvex. Later the cellulose wall of the spore becomes complete, but in just what way the author was not able to determine. The expanded end of the basidium shrinks and finally becomes drawn out to a point by the time the spore falls, but, contrary to Cornu, the tip still retains its callose plug and is not covered by a cellulose wall.

Reasoning by analogy, the author thinks the disarticulation of conidia in Peronosporæ takes place by a uniform mechanism. The paper is followed by a good lithographic plate (in part 5).

The following methods were employed to distinguish cellulose, callose, and protoplasm: Sections were first placed for some time in eau de Javelle* to remove plasmic matters. They were then washed in water and placed on slides with the addition of some drops of an alcoholic solution of soda or very concentrated caustic potash. After ten or twelve minutes they were neutralized with acetic acid and stained. Cellulose is colored a beautiful blue by a concentrated solution of iodated phosphoric acid. The stain is deep and instantaneous, the treatment with alkalies rendering the cellulose easier to stain. For callose one of the blues formed of trisulphonated triphenylrosaniline and soluble in water should be used. Since some nitrogenous matters may yet remain, it is well to mix one of these blues with a solution of acid brown (Bismarck, Vesuvin, etc). This mixture must always be used in an acid medium (acetic acid 3 to 100, formic acid 3 to 100). The cuticle and all azotic substances become brown, the cellulose remains colorless, and the callose becomes a brilliant greenish blue. After the action of this mixture, which requires some minutes, wash in water and mount in aqueous glycerine, in which the specimens will remain without bleaching for some months. Preparations treated with iodated phosphoric acid may be preserved in the same way and will keep for a long time if protected from the light.

In a footnote the author recommends the following dyes as especially serviceable: (1) For protoplasm, lignin, cutin, and pectin compounds: Blue de diphenylamine soluble in alcohol, blue de Bayer soluble in alcohol, bleu direct; bleu d'analine soluble in alcohol, bleu de gentiane 6B., bleu opal, bleu de nuit, bleu lumière. These blues do not stain callose. (2) For callose and protoplasm: Le bleu Nicholson 6B., le bleu soluble BLSE, le bleu coton C4B, from the house of Poirrier et Dalsace at St. Denis; le bleu brillant verdâtre pour coton, le bleu papier V. from Bayer et Cie at Flers near Roubaix; les bleus alcalins 6B, bleus nouveaux, G et R, from L. Cassella, Lyons; bleu de Bayer DBF, from Badische Analine Soda Fabrik, Neuville sur-Saône. These colors are soluble in water. They stain protoplasm a deep blue and callose a greenish blue; also lignin slightly. They do not stain cellulose. (3) For pectin compounds:

*Kaliumhypochlorite.

The azo acid browns, such as the Bismarck browns (Vesuvin, brun d' aniline). These do not stain cellulose or callose. (4) For protoplasm, lignin, and cutin: The acid browns of variable composition, often having no relation to Bismarck browns. These are salts of soda of which the coloring matter is the base. They are soluble in water. They stain protoplasm brown, and certain stain cellulose rose color, but feebly. They color lignin and cutin deeply in an acid bath. They do not stain pectin or callose compounds. They also mix with the soluble blues without precipitation and consequently are very suitable for the preparation of double stains, by means of which callose can be distinguished very readily in the midst of tissues rich in nitrogenous matters—ERWIN F. SMITH.

PECK, CHARLES H.—*Annual Report of the State Botanist of the State of New York.* Forty-fourth Rept. N. Y. State Mus. Nat. History: Albany, 1891, pp. 75, pl. 4.

The above was distributed to botanists during December, 1891, and is the most extensive contribution to systematic mycology issued during the year in this country. Prof. Peck continues his observations on fungi and gives descriptions of many new species, some of which are illustrated. In speaking of the liability of plants to the attacks of fungi, he says that certain species of spruce trees in a starved and unthrifty condition were attacked by *Peridermium decolorans*, while those in a healthy condition were exempt. The New York species of *Tricholoma* are monographed in a manner similar to genera in previous reports, forty-seven species being described. There is also given a notice of a manuscript volume by Mary E. Banning, which contains descriptions of some new species. The figures are colored by hand, and all the species were collected in Maryland. They are mostly Hymenomycetes and Gastromycetes. Fourteen new species are described.

The following is the contents of the report: (A) Plants added to the herbarium, including many species of fungi (pp. 9–11). (B) Contributors and their contributions (pp. 11–14). (C) Species of plants not before reported (pp. 15–30), with the following new species: *Armillaria viscidipes*, *Tricholoma grande*, *Clitocybe fuscipes*, *Collybia expallens*, *Omphalia corticola*, *Pleurotus pubescens*, *P. campanulatus*, *Flammula squalida*, *Crepidotus distans*, *Cortinarius albidus*, *Dædalea sulphurella*, *D. extensa*, *Hydnum arachnoideum*, *Odontea tenuis*, *Mucronella minutissima*, *Thelephora odorifera*, *Cyphella arachnoidea*, *Phyllosticta ludwigiæ*, *Dothiorella celtidis*, *Diplodia liriodendri*, *D. multicarpa*, *Septoria pteridis*, *Septomyxa carpini*, *Aspergillus aviarius* (found in the visceral cavity of a canary and supposed to have caused its death), *Sporotrichum Lecanii*, *Diplosporium breve*, *Ramularia destruens*, *R. junci*, *R. graminicola*, *Cercosporella veratri*, *Bispora effusa*, *Septonema episphæricum*, *Caryospora minor*, *Metasphæria nuda*, *Pseudopeziza pyri*, *Saccharomyces betulæ*, Pk. & Pat. (D) Remarks and observations (pp. 30–38) including